

SCREW-WORM ERADICATION PROPOSALS

eradicating
By ~~screw-worms~~, Callitroga hominivorax (Cqrl.) from the Island of Curacao^{as} in 1954 we proved the feasibility of screw-worm eradication solely through release of sterilized flies. Since the Curacao experiment, research has been directed toward developing plans and operational procedures for eradicating screw-worms from the southeastern states. The research has progressed to the point that definite recommendations can now be made.

It now appears practical to eradicate screw-worms from the southeastern states. This eradication would be accomplished primarily by releasing sterilized flies, but the chances of success for the program would be enhanced if, coupled with a fly release program, there would be a full-scale effort at securing farmer and rancher cooperation in reducing screw-worm breeding in livestock by use of insecticides and good animal husbandry practices.

Screw-worms in nature.

Screw-worms are obligatory parasites of warm-blooded animals, breeding only in wounds. They do not breed in carcasses. When an infested animal dies the older screw-worms that are nearly grown can survive but the younger maggots die in the carcass.

A single fly lays a mass of about 250 eggs which hatch within a day. The larvae feed for about 6 days. The 250 eat a hole in the flesh about as big as a lemon. Thus only fairly large animals are suitable as hosts. Common small animals such as rats are too small to live long enough for the maggots to finish their growth.

This biological fact limits the number of screw-worms in nature since only such wild animals as deer, foxes, raccoons, opossums, and rabbits are large enough to breed screw-worms. Further, infested wounds attract the eggs of other screw-worm flies so that wild animals are literally eaten alive in one or two weeks with only the larvae from the first egg masses having time to finish growing.

In ranching country probably more screw-worms breed in neglected domestic animals than in wild hosts. Cattle and hogs that roam in large pastures and are inspected infrequently breed screw-worms. Larvae from an egg mass finish growing and drop to the ground within one week after the eggs are laid. Thus a range cow may produce hundreds of screw-worms before its wound is treated with a remedy to kill the remaining larvae.

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The increase of screw-worms is rapid because the flies are prolific and the life cycle is short. Flies can mate when they are two days old and at six days of age a female can lay 200 eggs which hatch within a day. The larvae leave the wound after 6 days and drop to the ground. They crawl into the soil to pupate and after one week of summer weather emerge as adults. Thus in warm weather there can be a new generation every 3 weeks with a hundredfold increase each generation. In cool weather the pupal stage is prolonged and the adults take longer to develop their eggs for oviposition. In general, the average time from generation to generation may be about 4 weeks.

The screw-worm requires a tropical or subtropical climate for year-around survival. Frost kills the adults and the pupae cannot survive in cold ground. Prior to 193³ screw-worms did not occur in the southeast. A permanent infestation survived the winter in Mexico, the Rio Grande Valley of Texas, and in southern California. Each summer flies migrated several hundred miles northward.

The Texas infestation spread north as far as Kansas but never got far east of the Mississippi River. Each winter cold weather killed the flies back to the subtropical survival area. In the thousands of years of recent geological history, screw-worms never, by their own wing-power, reached the southeastern states.

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In 193², screw-worms appeared in Georgia apparently being shipped in with cattle from Texas. The infestation spread down into Florida and when winter came the insects were able to survive in Florida's subtropical climate.

Ever since, screw-worms have been destroyers of wild-life and livestock in the southeast, doing 5 to 15 millions of dollars of damage each year. They are active the year around in the area south of Orlando. During most winters screw-worms survive only in those areas warm enough to grow oranges. In average years the northern limit of winter survival is about on a line with Gainesville. In unusually mild winters screw-worms have survived in southern Georgia and the lower parts of Alabama and South Carolina. One very cold winter screw-worms were killed out to the latitude of Tampa. However, in average years screw-worms survive in the area south of Gainesville and Jacksonville.

A line drawn from St. Marks, Florida to Sea Island, Georgia is parallel to, and about 100 miles north of the usual winter survival boundary. The region south of this line represents about 50,000 square miles of potential screw-worm overwintering territory.

If screw-worms could be eradicated from this relatively small area, winter weather would kill them out of their summer range and all the southeastern states would be again free of screw-worm losses.

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Laboratory rearing of screw-worms.

Although in nature screw-worms can grow only in wounds in living animals, they are easily reared in the laboratory on an artificial medium. This nutritional medium is a mixture of finely-ground lean meat, citrated blood, and water with a small amount of formaldehyde added to retard putrefaction.

Larvae can be reared in any quantity desired in containers of a size appropriate to the amount of medium needed. To rear 200 larvae, a half-pint wide-mouth mason jar is used while 2,000 are produced in an aluminum bun pan or 100,000 in a special rearing pan 10 feet long, 3.5 feet wide and 1.5 inches deep.

The depth of the medium does not exceed 1.5 inches, regardless of the size of the container, because that is as deep as the larvae can reach to consume their food. In mass production, newly hatched ^{are} larvae/started on a shallower layer to prevent waste of meat. During the 5 days it takes the maggots to become full-grown they are given increasing quantities of fresh food at least 3 times as they cannot live in rotten meat as carcass maggots do.

The medium is kept heated at 95-100°F. to simulate the temperature of a wound.

The larvae feed as a colony in this medium. The maggots stay close together constantly feeding head-down with their posterior ends close to the surface of the semi-liquid medium. A maggot breathes through a pair of spiracles opening at the rear end

Laboratory rearing of saw-fly larvae

Although in nature saw-fly larvae can grow only in wounds in living animals, they are easily reared in the laboratory on an artificial medium. This nutritional medium is a mixture of finely-ground lean meat, sterilized blood, and water with a small amount of formaldehyde added to retard putrefaction. Larvae can be reared in any quantity desired in containers of a size appropriate to the amount of medium needed. To rear 300 larvae, a half-pint wide-mouth mason jar is used with 2,000 cc. of medium in an aluminum pan or 100,000 in a special rearing pan 10 feet long, 3.5 feet wide and 1.5 inches deep. The depth of the medium does not exceed 1.5 inches, regardless of the size of the container, because that is as deep as the larvae can reach to consume their food. In mass production, newly hatched larvae are started on a shallower layer to prevent waste of meat. During the 5 days it takes the maggots to become full-grown they are given increasing quantities of fresh food at least 3 times as they cannot live in rotten meat as voracious maggots do. The medium is kept heated at 95-100°F. to stimulate the temperature of a wound. The larvae feed as a colony in this medium. The maggots stay close together constantly feeding head-down with their posterior ends close to the surface of the semi-liquid medium. A maggot breathes through a pair of spiracles opening at the rear end

which it projects just through the surface of the medium to reach the air.

After 5 days when the maggots are grown they quit feeding and crawl about on the surface of the medium and fall over the edge of the container into a tray of sand. They burrow into this sand and pupate within a few hours of leaving the food.

The sand is sifted to remove the pupae which are then stored in trays at a temperature of 80°F. and a relative humidity of 100 percent. Within the dried, dark brown larval skin (puparium) the maggot becomes a fly. At 80°F. the transformation takes place in 7 days.

Just before the pupal stage is completed the insects are transferred to fly cages for emergence. A disposable cage of paper board, about one foot high, one foot wide and two feet deep holds 500 flies. The pupae for these flies are placed in an open paper cup in the cage along with a supply of water and a mixture of ground meat and honey which serves as fly food. The cages are held at 80°F. and 80 percent relative humidity.

When the flies are 2 days old mating begins and by 4 days practically all the females are mated. On the fourth day the honey-meat mixture is replaced by a dish of honey alone, mixed with oat hulls so the flies do not get trapped in it. At 6 days of age the females are ready to oviposit but maximum egg production is secured by waiting until they are 8 days old.

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The sand is sifted to remove the pupae which are then stored in trays at a temperature of 30°F . and a relative humidity of 100 percent. Within the third, dark brown larval skin (puparium) the maggot becomes a fly. At 80°F . the transformation takes place in 7 days.

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There are many ways to induce the flies to oviposit; but the easiest is to use a dish of rearing medium in which screw-worms have grown. A waxed paper cup, half filled with worm medium, held in a quart container in water heated to 105°F. is placed in the cage of 8-day-old flies. The females flock down to the medium and within an hour have laid their eggs, covering the inside of the cups, above the medium, with a layer of egg masses.

The eggs are scraped out of the oviposition cups and placed on moist paper toweling held in covered dishes at 80°F. In 24 hours the eggs have hatched and the larvae are placed on rearing medium.

Some of the flies would lay again if cared for another week; but the second egging does not justify the cost of holding the flies. Therefore, when oviposition is finished and the eggs are removed, the fly cage and its contents are incinerated.

There are many ways to induce the flies to oviposit; but the easiest is to use a dish of roasting medium in which scree-worms have grown. A waxed paper cup, half filled with warm medium, is placed in a quart container in water heated to 105°F. is placed in the cage of 3-day-old flies. The females flock down to the medium and within an hour have laid their eggs, covering the inside of the cups, above the medium, with a layer of egg masses. The eggs are scraped out of the ovipositor cups and placed on moist paper toweling held in covered dishes at 80°F. In 24 hours the eggs have hatched and the larvae are placed in roasting medium.

Some of the flies would lay again if caged for another week; but the second egg-laying does not justify the cost of holding the flies. Therefore, when oviposition is finished and the eggs are removed, the fly cage and its contents are incriminated.

Efficiency of laboratory propagation.

Laboratory conditions are controlled so that the insects are propagated in an optimum environment. They are protected from predation and disease. They are provided with food and water and held at the temperatures and humidities found most favorable for maximum survival, rapid growth and greatest increase.

In nature the biotic potential only equals the environmental resistance; so that, from year to year, the population remains stationary. However, in the laboratory, adverse factors are at a minimum so that ^{it} is feasible to rear, in a short time, and maintain this propagation of almost any numbers desired for a sterilized fly program.

The rate of propagation is illustrated with typical data based on averages recorded in recent experiments at the Orlando, Florida, laboratory.

A female fly lays 264 eggs. Within 24 hours, 95% hatch to produce 250 larvae. These are placed on the nutritional medium and 80% survive to produce 200 grown larvae after 4.5 days. Pupation occurs in 24 hours with 95% forming 190 pupae. After 7.5 days in the pupal stage 95% emerge to produce 180 flies (90 males and 90 females). The flies mate at 2 to 4 days and 90% of the females survive to oviposit when 8 days old. This egg production is 81×264 or 21,384.

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The complete generation required a total of only 22 days, with time spent in the various stages as follows: egg-1.0; larva-4.5; prepupa-1.0; pupa-7.5; adult (preoviposition)-8.0.

Each 22 days there is an 81-fold increase in population so only 44 days after the first female oviposits there are 891 females laying eggs. At 66 days there would be 72, 171; at 88 days 5,845,851, and at 110 days 473,513,931 females plus an equal number of males.

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These figures are theoretical but 20 years of rearing experience with several successes at rearing million-fly cultures prove that they are also practical of attainment. As shown earlier the biotic potential of screw-worm flies allows for an increase of 132 times per generation. Laboratory rearing conditions have been devised to minimize losses due to environmental resistance; so that under practical large-scale rearing conditions an 81-fold increase has been recorded. This degree of success might not be attained under conditions of mass rearing for an eradication campaign. However, the data are based on practical trials of rearing screw-worms at the rate of a million per week.

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Thus, once the breeding stock is built up; 2 percent of the fly production is returned to the colony for maintenance of breeding stock and 98% is available for sterilization.

Why sterilized flies can be used to eradicate screw-worm.

The foregoing sections dealt with these key points about screw-worm biology:

1. There are relatively few screw-worm flies per square mile in Florida because the larvae grow only in wounds in living warm-blooded animals.

2. Screw-worms had never been known to occur in the Southeast prior to their accidental introduction through shipment of infested cattle from Texas in 1933.

3. Although screw-worms are native to tropical and subtropical North and South America and migrate northward from Texas each year, the flies never succeeded in getting to the Southeast under their own wing-power. If the species could be eradicated from the Southeast it probably could not return by its own flight since it never succeeded previously.

4. Screw-worms cannot survive cold weather and, in winters of average severity, are killed out of all the Southeast except peninsular Florida.

5. Although flies are restricted in their winter distribution to an area of warm climate, and are limited in their numbers by availability of wounds in suitable living animals; they can be reared in any desired quantities in the artificial climate of the laboratory by raising the maggots on a nutritional medium which simulates a wounded animal.

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Why sterilized flies can be used in arthropod control.

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Laboratory by raising the pupae on a suitable medium which stimulates

The Entomology Research Division through its research during the past seven years has devised and proven a new method of biological control which takes advantage of the facts outlined above.

Screw-worm flies can be sterilized by exposure to ionizing radiation. Either X-rays or gamma-rays can be used and either adults or pupae are susceptible to irradiation. The cheapest and easiest form of radiation to use is gamma radiation from cobalt - 60. The stage most easily sterilized is the pupa, after 5 days development at 80°F.

Flies from irradiated pupae do not live quite as long as normal insects; but in other respects they are similar. They fly, feed, and mate like normal insects.

Male screw-worm pupae are sterilized by a dose of 2,500 roentgens of gamma rays while the females are more resistant and require 5,000 r. Even at that dose the females are capable of laying a few eggs which do not hatch. A dose of 7,500 r. prevents egg production entirely and has no more effect on other life processes than the lower doses, so the 7,500 r. dose has been routinely used in field tests with sterilized screw-worm flies.

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routinely used in field tests with sterilized scout-worm flies.

Male flies will mate as many as 15 times if enough females are available. Female flies mate only once. If a normal female mates with a sterilized male she will not mate subsequently with a normal male and lays only infertile eggs.

When caged populations containing various proportions of sterilized and normal insects were egged, the mates of the normal females could be known by ^{not} mating whether or not their egg masses hatched. Since infertile egg masses were in the same proportion as the ratio of sterilized to normal males, the sterilized males were as successful as normal males in competing for mates.

When sterilized screw-worm flies were released on Curacao in 1954 at the rate of 100 males per square mile, they mated with about 15% of the wild flies as determined from hatch ^{and} records of egg masses collected ^{from} wounded goats. This 15 percent of sterile mating was not sufficient to cause a population decline. However, when the release rate was increased to 400 sterile males per square mile about half the wild females laid infertile eggs during the first generation following fly release. This caused such a reduction in the natural population that the succeeding generations were even more outnumbered by sterilized males which were regularly released at the rate of 400 per square mile per week.

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week.

From July 19 to August 8 half the Island was treated at the 100 male rate and half with 400 males per square mile. Beginning August 9, all the Island was subjected to weekly releases of 400 males. The last evidence of native fly activity was the collecting of an infertile egg mass on November 11. Releases were continued for two more months to assure eradication but as far as the data show eradication was attained in 4 months.

Curacao has an area of only 170 square miles while the Florida overwintering area is 300 times greater. However, at the start of the work Curacao had an average screw-worm density index of 15 egg masses collected per goat^{pen} per week. A few locations in Florida are known where screw-worms are equally or somewhat more abundant but the average density in peninsular Florida would be much less.

Also, on Curacao, eradication was accomplished solely through release of sterilized flies. There was no help from livestock growers in application of insecticides and adoption of good animal husbandry practices as screw-worms breed chiefly in neglected, half-wild goats. The cooperation of Florida livestock producers should lessen the wild fly breeding pressure.

The Florida problem is more difficult than Curacao eradication only because of the greater area to be treated. In terms of unit area, releasing 400 sterilized males per square mile per week, should be as successful in Florida as on Curacao.

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There are also difficulties of climate. In warmer than average winters screw-worms may survive as far as 200 miles north of the area marked out for fly release. If this should occur during an eradication program, the work would have to continue until a normally cold winter limited the infested area.

Also, one should not count on the dramatically rapid eradication that occurred on Curacao. Instead of reducing fly breeding to zero in 4 months of releases as was experienced in Curacao, a longer time for control should be expected. Therefore, the first winter's effort in Florida should not be counted on but allowance should be made for some flies to survive and spread north of the treatment area with the coming of spring.

In such an event eradication efforts should continue in the overwintering area with the hope of success the following winter. Therefore, although there is a chance of a successful one year operation the program should be planned for two years. There should be plans for continuation after that if normal winter weather does not occur.

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TECHNIQUES FOR MASS PRODUCTION OF STERILIZED FLIES

The procedures described in the following sections are projections of research experience. We have maintained screw-worm colonies in the laboratory ever since we developed the artificial propagation method 20 years ago. Most research purposes required production of only a few hundred or a few thousand flies per week. However, since 1952 we have performed field tests with sterilized flies which required sustained production of many thousands of flies weekly. During 1956 there were two series of "pilot plant" tests at Orlando for experience at rearing and sterilizing one million flies weekly.

Based on this experience we have estimated the space and equipment requirements for an establishment capable of continuous production of 50 million flies weekly. We have been assisted in these estimates by engineers and entomologists of the Army Chemical Center, Ft. Detrick, Maryland. However, it should be emphasized that several months of intensive research and development need to be done as a cooperative effort between entomologists and engineers to draw precise specifications for the sterilized fly plant. The following account is presented only as a reasonable estimate of requirements.

1. The adult colony.

The flies are kept in disposable cages made of paper board (of the same sort that is used to make shipping boxes for canned goods). A cage measures 1' high by 1' wide and is 2' deep. The top, sides, and back have 1/8" holes punched to provide ventilation.

TECHNIQUES FOR MASS PRODUCTION OF STERILIZED FLIES

The procedures described in the following sections are projections of research experience. We have maintained screw-worm colonies in the laboratory ever since we developed the artificial propagation method 20 years ago. Most research purposes required production of only a few hundred or a few thousand flies per week. However, since 1952 we have performed field tests with sterilized flies which required sustained production of many thousands of flies weekly. During 1956 there were two series of "pilot plant" tests at Orlando for experience at rearing and sterilizing one million flies weekly.

Based on this experience we have estimated the space and equipment requirements for an establishment capable of continuous production of 50 million flies weekly. We have been assisted in these estimates by engineers and entomologists of the Army Chemical Center, Ft. Detrick, Maryland. However, it should be emphasized that several months of intensive research and development need to be done in a cooperative effort between entomologists and engineers to draw precise specifications for the sterilized fly plant. The following account is presented only as a reasonable estimate of requirements.

1. The adult colony.

The flies are kept in disposable cages made of paper board (of the same sort that is used to make shipping boxes for canned goods). A cage measures 1' high by 1' wide and is 2' deep. The top, sides, and back have $\frac{1}{8}$ " holes punched to provide ventilation.

The front is closed with elastic wrapping paper which stretches sufficiently to permit introduction of small dishes.

A fly cage is placed in operation by folding the cardboard sides in proper position much like a shipping box is assembled. A waxed paper cup containing 4 ounces of fly food (30% honey mixed with 70% ground heart) is placed on the floor of the cage along with a filled water fountain made by inverting a 6 oz. paper cup over a paper saucer. Then a papier-mache flower pot, 4" in diameter and 6" high, with 550 fully developed pupae in the bottom and one crumpled paper towel stuck loosely inside and projecting 3 inches above the top, is introduced. The cage is then closed by fastening the "stretchy" paper over the front.

On the average about 90% of the pupae yield 500 healthy flies which crawl on the sides of the pot and on the paper towel to expand their wings and harden their bodies.

As the insects all had pupated within an 8 hour period, fly emergence takes place over the same interval.

The flies need no more attention until they are 4 days old. Then an attendant removes the dish of meat and honey (otherwise some of the females might waste eggs by ovipositing on that food) and replaces it with another paper cup containing 4 ounces of a mixture of 80% honey and 20% oat hulls. (The oat hulls keep the flies from getting stuck in the honey.)

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flies from getting stuck in the honey.)

The flies are left without further attention until they are 7 days old. Then an attendant takes the cage to another room for oviposition.

There is some mortality of flies during the 7-day preoviposition period. Also, a few of the surviving females will not oviposit. It can be expected that a cage at 7 days will have 200 females which will lay an average of at least 200 eggs each. (In recent tests the average was 264.)

To take care of larval and pupal mortality in subsequent rearing steps it is advisable to start each day's larval cultures with 12,000,000 newly hatched larvae for a final yield of 50,000,000 flies weekly. The eggs hatch almost 100% so the production requirement of the adult colony is 12,000,000 divided by 200 or 60,000 ovipositing females from 300 cages with 200 reproducing flies.

This requires that 300 cages be started each day and remain in the adult colony room 7 days. The cages are sufficiently rigid that they can be stacked on top of each other 6 rows high with 10 cages in a row in a wheeled aluminum rack 10'6" long. The lowest cages in the rack are 1' off the floor. A lever extension arrangement lifts the rack 2' so the attendants can take care of the flies without removing the cages from the rack.

Since one rack holds 60 cages and 300 cages are set up each day a space 2' wide and 55' long is required for each new culture of flies. The flies are held in the adult colony room until they

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Since one rack holds 60 cages and 300 cages are set up each day a space 2' wide and 25' long is required for each new culture of flies. The flies are held in the adult colony room until they

are 7 days old so 7 55' rows are required. The rows are 3' apart to give working room for the attendants and space to maneuver the racks when the cages have to be moved. At one side of the room there is a 10' wide work area.

Thus the adult colony uses a room 45' x 63' or 2835 sq. ft. of floor space. The arrangement is shown in Figure 1.

2. Eggs and newly-hatched larvae.

When the flies are 7 days old they are ready to oviposit. An attendant wheels a rack of 60 cages into an adjoining oviposition room. One wall of the work space near the entrance is specially lighted. An area 10' long and 4' high starting 3' above the floor has rows of fluorescent lamps. The rack is placed so that the light shines in the ventilation holes in the backs of the cages, attracting the flies away from the front, preventing their escape while the oviposition dish is inserted.

The oviposition dish is a wide-mouth pint mason jar filled 1" deep with warm rearing medium taken from the vats in which larvae are growing in the larval rearing room. The medium is kept warm because the jar rests in a pan containing 1 quart of warm water. A disc of wrapping paper fits around the jar and covers the pan, helping maintain the temperature and protecting the flies from falling in the water.

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The oviposition dish is a wide-mouth pint Mason jar filled 1" deep with warm rearing medium taken from the vats in which larvae are growing in the larval rearing room. The medium is kept warm because the jar rests in a pan containing 1 quart of warm water. A disc of wrapping paper fits around the jar and covers the pan, helping maintain the temperature and protecting the flies from falling in the water.

After the 60 oviposition dishes are in place an attendant ^{leaves} ~~wh~~ wheels the rack away from the work area and ~~lets~~ the insects to oviposit.

The flies flock down to the warm rearing medium and feed on it. Then they move to the side and lay eggs on the clean glass wall above the medium. The first flies glue their egg masses to the glass adjacent to the medium. Those following oviposit on the first egg masses and on the glass above them. After two hours the females have finished ovipositing and the rack of cages is wheeled back to the specially lighted work area and the oviposition dishes and water fountains are removed.

Then the cages with their contents spent flies, pupa dish and food sish are wheeled to the incinerator. To prevent fungus and bacterial disease attacking the adult colony we have specified where feasible - cheap, disposable materials to be burned after one use.

The eggs are scraped from the walls of the mason jars and are placed on moist paper toweling held in covered pyrex pie plates. The eggs are weighed on a balance and _____ grams (150,000 eggs) are spread over moist paper toweling on the bottom of a pyrex casserole. Each day's egg production requires 80 of these dishes - one for each larval rearing vat.

The glass lids are placed on the casseroles which are ~~sotred~~ on shelves in the oviposition room. The larvae hatch in 24 hours

and are taken to the larval rearing room.

The space requirements for the oviposition room are shown in Figure 2.

Larval Rearing Procedures:

The larvae are reared in heated aluminum vats measuring 4.5 feet wide, 9.5 feet long and 1.25 inches deep. Each vat has a capacity of 100,000 larvae. In the experimental work these containers have been placed on an enclosed frame which is heated with thermostatically controlled lamps.

To save space in the rearing room for mass production, the vat will be constructed with built-in heating elements similar to those used in electric frying pans. A thermostat will control the temperature so that the medium at the bottom of the container is heated to $102^{\circ} \pm 2^{\circ}\text{F}$. Thus the vats can be suspended from the ceiling, hung on pulley-operated chains in racks of four spaced one foot apart.

To maintain production of 50 million flies weekly will require the growing of 500 vats of larvae. The insects require 5 days to complete the larval stage but some time should be allowed for occasional delays in larval development such as might occur if interruptions in electric service should allow the medium to cool. Also time is required for cleaning, sterilizing and refilling the vats. Therefore, it is estimated that each culture of 100,000 larvae uses a vat one week. Routine production requires space for 500 vats.

In addition to the regular production for the eradication program some vats need to be available to the research entomologists who will

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Larval Rearing Procedures:

The larvae are reared in heated aluminum vats measuring 4.5 feet wide, 2.5 feet long and 1.15 inches deep. Each vat has a capacity of 100,000 larvae. In the experimental work these containers have been placed on an enclosed frame which is heated with thermostatically controlled lamps.

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be simultaneously trying to develop a more efficient rearing medium. For large scale trials of new media and to have a safe reserve 60 vats should be available making a total of 560 required.

These 560 vats in racks of 4 would be arranged in 7 double rows 10 vats long. Each day of the week a new culture would be started in one of the double rows. Of the 80 vats in a double row 72 would be needed for routine production and 8 would be available to the research entomologist. The production of all 80 would be assigned 2% to colony and 98% to sterilization.

The double rows of rearing vats will hang over larva traps shaped somewhat like an inverted V. Each trap will be 80 feet long and 12 feet wide and fitted together in 5-foot sections made of sheet aluminum. A trap will come to a 2.5 foot peak at the center and the sides will slope down to sand troughs 8" high and 1' wide built in 5' sections for ease of emptying.

When the larvae finish feeding they will crawl from the rearing pans, fall on the sloping sides of the larva traps and roll into the sand troughs from which they will be removed periodically.

The details of arrangement of rearing vats over the larva traps are shown in Figure 3. The floor space requirements for the seven double rows of devices within the larval rearing room is shown in Figure 4. The over-all dimensions of the rearing room are 115'6" wide x 134' long (area 15,477 sq. ft.)

Pupa handling.

Starting at 6 a.m. when the first rearing shift comes to work and ending at 10 p.m. when the second shift finishes, larvae will be brought to the pupa room in wheel barrows loaded with sand taken periodically from the larva traps.

The wheelbarrows will be dumped on a machine-agitated sifter which will separate the insects from the sand. (The sand will be returned to the larva room for reuse.)

Some of the insects will have pupated in the sand traps so the first step is to scatter the mixed culture of larvae and pupae in thin layers on hardware cloth sieves 2' x 3' held over slightly larger sand trays. The larvae crawl through the coarse screen which retains the pupae. The pupae are washed in tap water and transferred to holding trays labeled with the day and hour of separation. The sand trays are resifted at approximately 8 hour intervals to separate the insects as they pupate. A culture will complete pupation in 40 hours.

The trays for holding pupae have aluminum sides 1 inch high and the bottoms are of aluminum window screen 2' x 3'. These trays are stacked in air conditioned cabinets through which flows a gentle draft of air at 80°F. and 100% relative humidity.

After 120 to 128 hours in those aging cabinets the sex organs of the pupae have developed to the proper stage for sterilization.

Then the pupae are loaded into 3 liter aluminum sterilization canisters. Each canister holds at least 25,000 pupae in the 5" by 11" cylindrical irradiation chamber. The pupae are merely poured into the container through a 1.5" hole at the top. This hole is covered with a two inch square of gummed tape as a safety procedure to prevent spilling any insects in the irradiation room.

The timing of loading the irradiation chambers is critical. The pupae must be at least 120 hours old; otherwise the gamma rays would kill them rather than merely destroy their reproductive capacity. Pupae need a good supply of oxygen and they would suffocate if left long in the irradiation chamber. Hence, a container should not be loaded more than 30 minutes before irradiation.

Sterilization of pupae.

The canisters of pupae are placed on a conveyor belt which runs through an 8' long passage from the pupa room to a separate building - the irradiation room.

The floor plan of the irradiation room and adjacent security area is shown in Figure 5. This room was designed in consultation with the ARS Radiological Safety Officer who conferred with scientists at the Brookhaven National Laboratory, to develop specifications for the sterilization facilities.

The room has to be large in order to give the operators safe working distances. The roof must be high to avoid risk of backscatter of gamma rays when the ports of the sealed sources are opened to insert or remove canisters of pupae. The building has removable

Then the pupae are loaded into 3 liter aluminum sterilization containers. Each container holds at least 25,000 pupae in the 5" by

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panels in the walls in front of each cobalt-60 source so that a truck with chain hoist can put each unit on its pedestal at time of installation. The high ceiling over each source is also required so that the hoist will have room to lift a source off its pedestal to transport it to the outdoor safe-handling well if mechanical difficulties should ever require servicing of a cobalt-60 source.

The irradiation room measures 32' by 76'. The floor is of poured concrete and does not need to be specially constructed except at the base of each of the 4 cobalt-60 sources. These are mounted on 3' concrete pedestals on separate foundations each of which bear a 6-ton load. The walls of the building are of light-weight aluminum siding fastened to 2 x 4 wooden studs. At the front of the room over the sources the walls are 18' high. At the rear of the room the walls are 10' high. A shed roof of light weight aluminum on wooden rafters slopes from front to rear. Near the roof at front and rear louvered aluminum windows with aluminum screens provide light and ventilation. This is the only room in the building that does not require accurately controlled temperatures since the insects pass through in a few minutes. A gamma-ray source consists of plates of cobalt-60 (400 curies) mounted in stainless steel holders fastened in a 5-ton cylinder of lead and steel. The radio cobalt plates are arranged around a central well in which the 3-liter aluminum canister is inserted for irradiation.

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The cobalt-60 plates are so arranged that radiation of rather uniform intensity (1600 roentgens $\pm$ 10% per minute subjects all the pupae in the 3 liter volume to a sterilizing dose (8,000 or $\pm$ 10%) of gamma rays in 5 minutes exposure.

An operator can attend two sources. He picks a canister of pupae from the conveyor and fastens it on the insertion grapple of the canister handling broom. The grapple fits into the $1\frac{1}{2}$ " hole in the top of the canister (and breaks the tape which sealed it). Then the operator works a lever which opens the port to the irradiation chamber and swings the canister in position over the opening. A hydraulic lift lowers the canister into position in 4 seconds. Then the operator lifts out the grapple and closes the port. After 5 minutes he opens the port, inserts the grapple and lifts out the canister. He removes the canister and places it on the conveyor belt where it passes to the sterilized fly receiving room. The broken seal on the canister shows the operators in that room that the insects are safely sterilized.

(For those not familiar with radiation effects it is here mentioned that neither the insects nor the canisters become radioactive in the process of irradiation. The gamma rays which sterilize the insects are merely very short waves of energy much like those produced by an x-ray machine. The irradiated materials do not come in direct contact with the radioactive cobalt. They are only penetrated by high energy waves of radiation which emanate from the radio-cobalt.)

The scheduled daily production of 7,000,000 pupae for sterilization loaded 25,000 to the canister requires routine irradiation of 280 canisters. Assuming 10 minutes per operation (at 5 minutes in the irradiation well and 5 minutes for handling between loads) each 400 curie source could sterilize 96 canisters in a 16 hour day of two 8-hour shifts. Three sources could therefore sterilize the scheduled production if mechanical breakdown of one unit should put it out of production for a time. Four sources are specified as a margin of safety. A further margin of safety in case of mechanical difficulty or to handle increased pupal production could be attained by adding a night shift.

Since an operator gives no attention to a unit during the 5 minutes the canister is being irradiated he has that time to load and unload his other assigned source.

Handling sterilized pupae.

All the processes concerned with rearing the insects have to be conducted under conditions of special security to prevent any normal adults, grown larvae or pupae escaping from the fly-proof production building. However, after the canisters of pupae leave the irradiation room those special precautions can be relaxed. All that the operators in the sterilized fly receiving room have to do in the way of a safety measure is to make sure that canister safety seal is broken as proof that it was irradiated.

The scheduled daily production of 7,000,000 pupae for sterilization loaded 25,000 to the canister requires routine irradiation of 230 canisters. Assuming 10 minutes per operation (at 5 minutes in the irradiation well and 5 minutes for handling between loads) each 400 canister source could sterilize 96 canisters in a 16 hour day of two 8-hour shifts. Three sources could therefore sterilize the scheduled production of mechanical products of one unit should not it enter production for a time. Four sources are specified as a margin of safety. A further margin of safety in case of mechanical difficulty or to handle increased pupal production could be attained by adding a night shift.

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The insects still have to be cared for most carefully to assure that maximum numbers of strong, healthy sterile flies are released. The pupae still have two more days to develop at 80°F. and 100 percent relative humidity before the adults emerge. They can stand a temperature range of 60° to 95°F but the low temperature would delay emergence and the high temperature would hasten development. The temperature should be kept at 80°F to keep sterilized fly production on schedule. The pupae can also survive several hours at a relative humidity as low as 50% but they should be subjected to a variable humidity only while being transported. As many hours as possible during the last 48 hours of pupal life should be at a controlled relative humidity of 95 to 100%.

A special emergence cage has been designed to make it easy to load flies in the airplane release device. This cage is a V-shaped trough made of aluminum. The sides are 2" strips of sheet aluminum, 7'6" long. The top is of reinforced aluminum screen, 2" wide and 7'6" long with a 1" aluminum frame which is hinged to one side of the trough and fastens with snap locks to the other. The bottom of the trough is a strip of aluminum only 3" wide which is also hinged on one side and fastened with snap locks on the other.

Eleven rows of spring clips, attached 2" apart to the screen top, hold 11 strips of paper toweling which stretch the length of the cage and vary in width to just touch the sides and layer of pupae which will be placed in the bottom of the cage.

As soon as the pupae are received from the irradiation room the canisters are emptied in emergence cages at the rate calculated to yield 48,000 flies. Ordinarily two canisters will be the exact bad for a cage but if pupae are larger or smaller than average an adjustment will be made for size.

The scheduled daily production of 7,000,000 pupae loaded at 53,000 per emergence cage (to produce 48,000 flies) will fill 132 emergence cages. As a margin of safety and to take advantage of hoped for improvements in production, space for holding 200 emergence cages is required. Each cage fits into a shipping crate 2'2" wide, 2'6" high and 7'8" long. The crates are made higher than the cages to allow air circulation within stacks of crates.

These crates will be stacked in a shipping room air conditioned to maintain a temperature of 80°F and 95-100% relative humidity.

A row of 10 crates will require floor space of 8' by 24'. Stacked 4 crates high there will be 40 crates in a row. The rows should be spaced 4' apart to give room for handling and 4' from each ~~xi~~ aisle for ease of access. A days production fo 200 shipping crates will need floor space of 32' by 60'.

Bad weather or some other emergency might not permit shipping ~~the~~ ^{the} in insects within 24 hours of sterilization so double storage space or an area 60' x 60' should be air conditioned to hold two days' maximum production.

The fly storage building will be a separate unit equipped with a roofed loading dock, sothe pupae can be put in delivery trucks without getting wet during rains.

As soon as the pupae are received from the irradiation room the containers are emptied in emergency cages at the rate calculated to yield 18,000 flies. Ordinarily two containers will be the exact bed for a cage but if pupae are larger or smaller than average an adjustment will be made for size.

The scheduled daily production of 7,000,000 pupae loaded at 15,000 per emergency cage (to produce 18,000 flies) will fill 125 emergency cages. As a margin of safety and to take advantage of hoped for improvements in production, space for holding 200 emergency cages is required. Each cage fits into a shipping crate 24" wide, 24" high and 7'8" long. The crates are made higher than the cages to allow air circulation within stacks of crates.

These crates will be stacked in a shipping room air conditioned to maintain a temperature of 80°F and 92-100% relative humidity.

A row of 10 crates will require floor space of 3' by 24'. Stacked 4 crates high there will be 40 crates in a row. The rows should be spaced 4' apart to give room for handling and 4' from each end aisle for ease of access. A days production of 200 shipping crates will need floor space of 32' by 60'.

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The fly storage building will be a separate unit equipped with a roofed loading dock, where pupae can be put in delivery trucks without getting wet during rains.

Before the flies emerge (at any time within 48 hours of irradiation) the insects will be shipped via air conditioned truck to the distribution points. There will be 15 distribution points, each at a strategically located airport. All will be within 8 hours drive of the sterilized fly production center.

The average daily shipment to a distribution point will be 9 cages but reserve space should be available when pupa production exceeds the quota or when it is desired to treat an area with more than 400 sterilized males per square mile. Therefore shipping space should be allowed for 12 cages to a distribution point. Five trucks will be dispatched daily, each delivering to three distribution points. Delivering 12 crates to each of 3 points requires space for 36 crates.

If the crates are laid crosswise on the truck bed in a row 12 crates long and stacked 3 crates high, they need a minimum space ~~two 36 footers~~ 8' wide, 28' long and 8' high. The van should

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As the five trucks will need one day to deliver their loads and another day to return, ten trucks will be in daily use. Two more trucks should be in stand-by status, available in case of breakdown or to deliver extra flies to centers selected for specially large fly release. Therefore fly distribution requires 12 specially air conditioned trucks.

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As the five trucks will need one day to deliver their loads and another day to return, ten trucks will be in daily use. Two more trucks should be in stand-by status, available in case of breakdown or to deliver extra flies to centers selected for especially large fly release. Therefore fly distribution requires 12 specially air conditioned trucks.

Since 200 emergence cages will be loaded on the fifth day of pupal development and flies do not emerge until they have completed 7 days in the pupal stage, 600 emergence cages will be needed for pupae. The adult flies will be routinely held in the emergence cages for 24 hours before they are transferred to airplane release devices. In case of bad flying weather the flies might be kept in the emergence cages as long as 3 days. Therefore, 600 cages should be allocated for holding adults. When emptied the cages would be in transit one day and in the cleaning room a second day for washing and sterilizing. Thus 400 cages would be required for the 2-day return period. The total requirement is 1400 emergence cages.

STERILIZED FLY DISTRIBUTION POINTS

Each of 15 fly distribution points will be manned by a GS-7 entomologist and a GS-3 biological aid who will receive daily shipments of pupae, hold them under proper conditions, care for the flies after they emerge; and load the airplane distribution devices with CO₂ anesthetized flies. (In addition they will have other duties connected with technical field information to be described later.)

1. Pupa handling.

The over age daily shipment received at a distribution point will be 9 cages of pupae each yielding 48,000 flies but areas where eradication is especially difficult may receive daily shipments of as many as 18 cages.

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1400 emergence cages.

STERILIZED AIR DISTRIBUTION POINTS

Each of 15 air distribution points will be manned by a GS-7

entomologist and a GS-3 biological aid who will receive daily

shipments of pupae, hold them under proper conditions, care for

the flies after they emerge, and load the airplane distribution

devices with 500 anesthetized flies. (In addition they will have

other duties connected with technical field information to be

described later.)

1. Personnel

The over age daily shipment received at a distribution

point will be 9 cages of pupae each yielding 48,000 flies but areas

where eradication is especially difficult may receive daily shipments

of as many as 18 cages.

The distribution point will have a two room insectary. A pupa holding room will be air conditioned to 80°F. At one end wheeled racks 2'6" wide with space for 3 cages one above the other, 6" apart, will be arranged. Six racks, each 8' long will occupy floor space 18' x 10'. A humidifier mounted over the racks will keep the humidity close to 100%. A plastic curtain extending from ceiling to floor will permit a lower humidity in the adjoining 18' x 10' work area.

Pupae will be held in the curtained area for a variable time depending upon how much of the 48 hours of pupal development after sterilization remains when they are delivered to the distribution point. When fly emergence begins the racks of pupae will be wheeled into the adjoining adult room.

2. Adult handling.

The adult room will be without windows so that flies can be held in darkness if bad flying weather or airplane trouble prevents their scheduled release. To have room for holding the "pile-up" of cages in such circumstances, the adult room will measure 36' x 20' with room for 36 cages.

The room will be routinely air conditioned at 80°F and 50% relative humidity but there will be reserve refrigeration power to reduce the temperature to 65°F in the event that fly releases are delayed.

The cages of emerging flies will be left undisturbed overnight. Early the next morning the ceiling lights in the adult room will be

The distribution point will have a two room laboratory. A pupa holding room will be air conditioned to 80°F. At one end thereof racks 2'6" wide with space for 3 cages one above the other, 6" apart, will be arranged. Six racks, each 8' long will occupy floor space 18' x 10'. A humidifier mounted over the racks will keep the humidity close to 100%. A plastic curtain extending from ceiling to floor will permit a lower humidity in the adjoining 18' x 10' work area.

Pupae will be held in the contained area for a variable time depending upon how much of the 48 hours of pupal development after sterilization remains when they are delivered to the distribution point. When fly emergence begins the racks of pupae will be wheeled into the adjoining adult room.

2. Adult handling.

The adult room will be without windows so that flies can be held in darkness if bad flying weather or airplane trouble prevents their scheduled release. To have room for holding the "pile-up" of cages in such circumstances, the adult room will measure 36' x 50' with room for 36 cages.

The room will be routinely air conditioned at 80°F and 50% relative humidity but there will be reserve refrigeration power to reduce the temperature to 65°F in the event that fly releases are delayed.

The cages of emerging flies will be left undisturbed overnight. Early the next morning the ceiling lights in the adult room will be

turned on, attracting the flies to the top of the cage. Then the hinged 3" wide bottom will be opened so that the empty pupal cases fall out to free the cage of this debris. Then the bottom is snapped back in place to prevent the flies escaping.

Next the flies are given a meal of sugar syrup by pouring the liquid in thin "strings" over the screen top. Enough of the syrup is given so that it takes the flies 30 minutes to clean the screen.

When the airplane pilot is ready for a load of flies a rack of cages is wheeled into the work area of the pupa room. A plastic cloth is laid over the top of the cage and carbon dioxide gas is released under the cloth through a hose connected to a CO₂ tank. Within a minute the flies fall to the bottom of the cage. Then the cloth is removed, the screen top opened and paper towels are pulled from the clips and discarded. The top is again closed and covered once more with the plastic cloth. Another whiff of CO₂ is given to knock out the reviving flies.

Then the cage is placed on a special rack over the airplane release device which is 7'6" long, and is divided into a double row of compartments each 6" square and $\frac{1}{2}$ " wide. The worker opens the top of the cage and while his assistant lets a little CO₂ hiss over the flies, with his hand he gently spreads the 48,000 anesthetized flies in a uniform layer over the 7'6" long bottom panel and the flies fall into the compartments of the release device. He fastens a flexible brass ribbon in place to lock the flies in their compartments and turns the device over to fill the other side in a

turned on, attaching the flies to the top of the cage. Then the

hinged 3" wide bottom will be opened so that the empty pupae

cases fall out to free the cage of this debris. Then the bottom

is snapped back in place to prevent the flies escaping.

Next the flies are given a meal of sugar syrup by pouring the

liquid in this "syringe" over the screen top. Enough of the syrup

is given so that it takes the flies 30 minutes to clean the screen.

When the airplane pilot is ready for a load of flies a rack of

cages is wheeled into the work area of the pupa room. A plastic

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cloth is removed, the screen top opened and paper towels are pulled

from the clips and discarded. The top is again closed and

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release device which is 7'6" long, and is divided into a double row

of compartments each 6" square and 1/2" wide. The worker opens the

top of the cage and while his assistant fans a little CO₂ gas over

the flies, with his hand he gently spreads the 48,000 anesthetized

flies in a uniform layer over the 7'6" long bottom panel and the

flies fall into the compartments of the release device. He fastens

a flexible brass ribbon in place to lock the flies in their

compartments and turns the device over to fill the other side in a

similar manner.

When a second device is loaded the insects are ready for distribution. The flies must then be promptly taken to the airplane for immediate take-off because they are crowded in their compartments and would soon suffocate without the air which circulates through the devices when the airplane is in flight.

AIRPLANE DISTRIBUTION OF STERILIZED FLIES

All of our research experience on air distribution of flies has been in treating rather limited areas. Curacao had an area of only 170 square miles. The largest area treated in Florida so far has been south of Lake Okechobee where for 6 weeks we put sterilized flies out over two 1000 square mile plots at 400 males (and 400 females) per square mile per week.

For those experiments an entomologist rode with the pilot and dropped the flies in paper containers which he opened just before throwing them out of the airplane. This procedure should be used only as a last resort in an eradication program. More man-hours are needed to package the flies in small containers than are required for the whole rearing process. The weight of the entomologist in the airplane reduces the pay-load that the plane could carry if the pilot flew alone and released the flies automatically from a mechanically operated dispersing device.

The problems of fly dispersal have been discussed with engineers of the Aircraft and Special Equipment Center, Plant Pest Control Division. That organization has worked on the design of a

similar manner.

When a second device is loaded the insects are ready for distribution. The flies must then be promptly taken to the airplane for immediate take-off because they are crowded in their compartments and would soon suffocate without the air which circulates through the device when the airplane is in flight.

ATMOSPHERIC DISTRIBUTION OF STERILIZED FLIES

All of our research experience on air distribution of flies has been in treating rather limited areas. Curacao had an area of only 170 square miles. The largest area treated in Florida so far has been south of Lake Okechobee where for 6 weeks we put sterilized flies out over two 1000 square mile plots at 400 miles (and 400 females) per square mile per week.

For these experiments an entomologist rode with the pilot and dropped the flies in paper containers which he opened just before throwing them out of the airplane. This procedure should be used only as a last resort in an eradication program. More man-hours are needed to package the flies in small containers than are required for the whole rearing process. The weight of the entomologist in the airplane reduces the payload that the plane could carry if the pilot flew alone and released the flies automatically from a mechanically operated dispensing device.

The problems of fly dispersal have been discussed with engineers of the Aircraft and Special Equipment Center, Plant Test Control Division. That organization has worked on the design of a

dispersing device and developed flight plans for releasing flies over the eradication area with light airplanes equipped with automatic fly dispersers.

The device which has been designed (but not yet built and tested) was mentioned in the foregoing section under the description of loading flies at a distribution point. It consists of two 6" x 6" square channels each made of two 7'6" strips of sheet aluminum and fastened to a 1" x 6" duct 7'6" long and terminating in an "air scoop". Aluminum screen keeps flies from getting in the duct and permits air to flow into the channels which are divided into $\frac{1}{2}$ " compartments. A flexible thin brass belt 6" wide stretches all around the device locking in the flies. The belt moves on an electrically powered drive wheel and has one slot measuring $1\frac{1}{2}$ " x 4" which passes over the compartments as the wheel moves the belt. This lets air rush through a compartment to blow the flies out through the belt opening.

A small sectional model of this device was tested in a wind tunnel at Fort Detrick, Maryland, and it looks promising. The screw-worm flies survived being blown out without injury. It now is necessary to build full-size devices to perfect the equipment for an eradication effort. The plans for releasing sterilized flies are based on the expectation that the Plant Pest Control Division will develop the release device in time for the eradication campaign. If such an automatic mechanical device is not developed and it becomes necessary for a fly man to ride with the pilot, throwing out the insects in paper containers, as has been done in our research, annual costs of fly distribution will be at least double the \$500,000 now estimated.

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Aluminum screens keep flies from getting in the duct and permits

air to flow into the channels which are divided into 1/2" compartments.

A flexible thin brass belt 6" wide stretches all around the device

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our research, annual costs of fly distribution will be at least

TECHNIQUES FOR MASS PRODUCTION OF STERILIZED FLIES

The procedures described in the following sections are projections of research experience. ~~We have maintained~~ ^{have been maintained} Screw-worm colonies in the laboratory ever since ~~we developed~~ the artificial propagation method was

^{developed} 20 years ago. Most research purposes required production of only a few hundred or a few thousand flies per week. However, since 1952 ~~we~~ ^{have been conducted} ~~have performed~~ field tests with sterilized flies which required sustained production of many thousands of flies weekly. During 1956

there were two series of "pilot plant" tests at Orlando for experience at rearing and sterilizing one million flies weekly. ^{This level of production, although high, is still}

^{a fairly accurate estimate has been made of space, equipment and personnel requirements for an establishment capable of continuous production of 50 million flies weekly. ^{a minimum of} ~~We have been assisted in~~ ^{assistance has been} ~~these estimates~~ by engineers and entomologists of the Army Chemical Center, Ft. Detrick, Maryland. ^{in making these estimates.} However, It should be emphasized that several months of ^{operational} ~~intensive~~ research and development need to be done as a cooperative effort between entomologists and engineers to draw precise specifications for the sterilized fly plant. ^{both in vision and regulatory work would} ~~Permit more precise estimates of requirements for the construction and operation of~~ ^{information can be regarded} ~~is presented only~~ as a reasonable estimate of requirements.}

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^{operational} ~~intensive~~ research and development need to be done as a cooperative effort between entomologists and engineers to draw precise specifications for the sterilized fly plant. ^{both in vision and regulatory work would} ~~Permit more precise estimates of requirements for the construction and operation of~~ ^{information can be regarded} ~~is presented only~~ as a reasonable estimate of requirements.

1. The adult colony ^{brood} ~~facilities~~ ^{and maintenance}

The flies are kept in disposable cages made of paper board (of the same sort that is used to make shipping boxes for canned goods). A cage measures 1' high by 1' wide and is 2' deep. The top, sides, and back have 1/8" holes punched to provide ventilation.

area in Florida can obviously lead to more complications. However, if the same procedures are projected to the larger area the results can be expected to be the same. At the start of the work, Curacao had an average screw-worm density index of 15 egg masses collected per goat *per* week. *Screw worms are equally or more abundant in* Some locations in Florida are known where screw worms are ~~equally or somewhat more abundant~~ but normally the average density in peninsular Florida is regarded to be no greater than that which occurred on Curacao. Currently the natural population in Florida is unusually high. The release rate of an average of 400 sterile males per square mile per week is therefore recommended as a minimum for the Florida program under consideration, *and efforts should be made to increase this level by achieving maximum efficiency in production and handling the flies.*

9. 8. On Curacao, eradication was accomplished solely through release of sterilized flies. There was no help from livestock growers in application of insecticides and adoption of good animal husbandry practices as the screw-worms bred chiefly in neglected, half-wild goats. If the cooperation of Florida livestock can be obtained to the extent hoped for the fly breeding pressure should be lessened with resulting increase in effectiveness of the sterile males.

10 9. In warmer than average winters screw-worms may survive as far as 200 miles north of the area marked out for fly release. *If In the event* ~~this should occur during an eradication program~~, the work would have to continue until a normally cold winter destroys the infestation *or complete or near eradication* north of the eradication zone. *If eradication has been achieved in the*

How to cope with such situation can best be determined by considering the overall situation in and outside of the eradication zone at that time.

area in Florida can obviously lead to more complications. However, if the same procedures are projected to the larger area the results can be expected to be the same. At the start of the work, Curacao had an average sero-worm density index of 15 egg masses collected per host per week. Some locations in Florida are known where sero-worms are equally or somewhat more abundant but normally the average density in peninsular Florida is regarded to be no greater than that which occurred on Curacao. Currently the natural population in Florida is unusually high. The release rate of an average of 400 sterile males per square mile per week is therefore recommended as a minimum for the Florida program under consideration, and efforts should be made to increase this by releasing more males during the breeding season.

On Curacao, eradication was accomplished solely through release of sterilized flies. There was no help from livestock growers in application of insecticides and adoption of good animal husbandry practices as the sero-worms had chiefly in neglected, half-wild goats. If the cooperation of Florida livestock can be obtained to the extent hoped for the fly breeding pressure should be lessened with resulting increase in effectiveness of the sterile males.

In winter, when average winter sero-worms are native as far as 200 miles north of the area marked out for fly release, it is thought that during an eradication program, the work would have to continue until a normally cold winter destroys the infestation north of the eradication zone. All eradication has been achieved in the

extended

possible

original

eradication zone, the release of sterile males should be moved northward to the extent possible, but release of sterile males should continue in the original eradication zone at a rate that will prevent re-establishment and build up from the infested area. The dramatically rapid eradication that occurred on Curacao ^{can hardly be} should not be expected in Florida. Instead of reducing fly breeding to zero in 4 months of releases as was experienced in Curacao, a longer time for control should be expected. ^{achieving the objective} Moreover, the first winter's effort in Florida may not reduce the population fast enough to prevent the northward spread ^{from} out of the eradication zone. ~~to survive and spread north of the treatment area with the coming of spring.~~

Suggest
S. project
1 + 1
no 11

In such a large area as Florida, pockets of infestations might persist for some months even if the overall population had declined ~~to nearly zero~~

delete

In such an event eradication efforts should continue uninterrupted in the eradication zone with the ^{expectation} hope of success during the following year. ^{although} Therefore, there is reason to hope for a success in one years operation, the program should be planned for at least two years. There should be provisions for continuation after that, if normal winter weather does not occur.

Bush I believe item 11 ^{above} should follow item 9., thus making current item 10 the last and no 11.

On further thought I believe the current item 10 could be eliminated here since you will no doubt discuss the eradication procedure in detail elsewhere.

(1)

Larvae Rearing Procedures:

The larvae are reared in aluminum trays measuring 4.5 feet wide, 9.5 feet long and 1.25 inches deep. Each tray has a capacity of 100,000 larvae. In the experimental work the trays have been placed on an enclosed frame which is heated with thermostatically controlled lamps.

To save space in the rearing room for mass production, the trays will be constructed with built-in heating elements similar to those used in electric frying pans. A thermostat will control the temperature so that the medium at the bottom of a tray is heated to $102^{\circ} \pm 2^{\circ}\text{F}$. Thus the trays can be suspended from the ceiling, hung on pulley-operated chains in racks of four trays spaced one foot apart.

To maintain production of 50 million flies weekly will require the growing of 500 trays of larvae. The insects require 5 days to complete the larval stage but some time should be allowed for occasional delays in larval development such as might occur if interruptions in electric service should allow the medium to cool. Also time is required for cleaning, sterilizing and refilling the trays. Therefore, it is estimated that each culture uses a tray a week. Routine production requires space for 500 trays.

In addition to the regular production for the eradication program some trays need to be available to the research entomologist who will be simultaneously trying to develop a more efficient rearing medium.

Large Rearing Procedures:

The larvae are reared in aluminum trays measuring 4.5 feet wide,

9.5 feet long and 1.25 inches deep. Each tray has a capacity of

100,000 larvae. In the experimental work the trays have been placed

on an enclosed frame which is heated with thermostatically controlled

lamps.

To save space in the rearing room for mass production, the trays

will be constructed with built-in heating elements similar to those

used in electric drying pans. A thermostat will control the tem-

perature so that the medium at the bottom of a tray is heated to

$102^{\circ} \pm 2^{\circ}F$. Thus the trays can be suspended from the ceiling,

hung on pulley-operated chains in racks of four trays spaced one

foot apart.

To maintain production of 50 million flies weekly will require

the growing of 500 trays of larvae. The insects require 5 days to

complete the larval stage but some time should be allowed for

occasional delays in larval development such as might occur if

interruptions in electric service should allow the medium to cool.

Also time is required for cleaning, sterilizing and refilling the trays.

Therefore, it is estimated that each culture uses a tray a week.

Routine production requires space for 500 trays.

In addition to the regular production for the eradication program

some trays need to be available to the research entomologist who will

be simultaneously trying to develop a more efficient rearing medium.

(2)

For large scale trials of new medium and to have a safe reserve 60 trays should be available making a total of 560 trays required.

These 560 trays in racks of 4 would be arranged in 7 double rows 10 trays long. Each day of the week a new culture would be started in one of the double rows. Of the 80 trays in a double row 72 would be needed for routine production and 8 would be available to the research entomologist. The production of all 80 trays would be assigned 2% to colony and 98% to sterilization.

The double rows of rearing trays will hang over larva traps shaped somewhat like an inverted V. Each trap will be 80 feet long and 12 feet wide and fitted together in 10 foot sections made of galvanized sheet metal. A trap will come to a 2.5 foot peak at the center and the sides will slope down to sand troughs 6 " high and 1' wide.

When the larvae finish feeding they will crawl from the rearing pans, fall on the sloping sides of the larva trap and roll into the sand trough from which they will be sifted periodically.

For large scale trials of new medium and to have a safe reserve 60 trays should be available making a total of 500 trays required. These 500 trays in rooms of 4 would be arranged in 7 double rows of 10 trays each. Each day of the week a new culture would be started in one of the double rows. Of the 80 trays in a double row 72 would be needed for routine production and 8 would be available to the research entomologist. The production of all 80 trays would be assigned 25 to colony and 55 to sterilization. The double row of rearing trays will hang over jars trays shaped somewhat like an inverted V. Each tray will be 80 feet long and 12 feet wide and fitted together in 16 foot sections made of galvanized sheet metal. A trap will come to a 2.5 foot peak at the center and the sides will slope down to sand troughs 6" high and 1' wide.

When the larvae finish feeding they will crawl from the rearing pans, fall on the sloping sides of the jars trap and roll into the sand trough from which they will be lifted periodically.

How are pupae packaged
and how best for emergency -

are flies to be fed -
stock flies + pupae -
-18-

One experience with this new equipment may suggest certain changes but modifications should be minor. Each unit will be operated 24 hours a day and will have a sterilizing capacity in excess of 10,000,000 pupae per week. On the basis of bids for construction of source housings and Brookhaven's charge for the radiocobalt, the units should cost \$7,000 each or \$35,000.

Sterilizing the flies is one of the simplest and least expensive steps in the whole eradication program.

Airplane distribution of sterilized flies.

For the Curacao experiment pupae were reared and irradiated at Orlando, Florida. They were then packed in groups of 130 (expected fly emergence - 100) in No. 1 kraft paper sacks and shipped by air freight to Curacao where the flies emerged. The newly emerged flies were distributed over the Island by airplane. An entomologist rode with the pilot, tearing open the sacks and dropping the insects at desired intervals.

The airplane covered the Island twice weekly flying one mile swaths. On each flight the insects were dropped at the rate of 200 per linear mile. On alternate flights the flight lanes were shifted 1/2 mile. Thus the practical effect was that the insects were put out in half-mile swaths at the rate of 400 per sq. mile per week.

In experiments on the mainland of Florida, paper sacks, paper cups and frozen food cartons have been used as containers. In all the tests an entomologist has ridden with the pilot to distribute the flies. This procedure has worked well for field tests but is too laborious for an eradication program. Also the weight of the entomologist reduces the pay load ^{for} of sterilized insects *and space for the containers.*

It seems essential to develop a device for automatically releasing the flies. This problem has been referred to the Aircraft and Special Equipment Center, Plant Pest Control Division. That organization has developed preliminary plans for automatic devices which can be attached to an airplane somewhat like wing tanks, which will release the flies automatically in desired numbers at regular intervals. This work is being aided by joint planning and testing in cooperation with specialist of the Biological Warfare Research group at Ft. Detrich, Maryland.

It seems certain that the Aircraft and Special Equipment Center can in a few months develop suitable release devices.

Recent experiments in Florida suggest that distributing flies in half-mile swaths each week as was done on Curacao represents closer distribution than is necessary to assure adequate dispersal of the sterilized insects. The Aircraft Equipment Center has worked out flight plans for treating the Florida overwintering area. By this plan an airplane covers an assigned area flying 3-mile swaths. Each day the flight is repeated with the swath moved one mile. By the third day the area is covered in mile swaths. Flights are repeated the last 3 days of the week giving essentially the same coverage as on Curacao. It is believed that the daily swath

width can be increased to 4.5 miles with moves of 1.5 miles on each successive flight, thereby reducing airplane distribution costs one-third.

Airplane dispersal over Florida represents the major problem still not adequately answered by research and development. It seems certain that with a few months intensive effort that efficient and economical methods can be developed to greatly reduce dispersal costs.

Production and distribution of sterilized flies.

For current experiments flies are reared and sterilized at Orlando, Florida, because of the convenience of the location and because various laboratory and field tests are being conducted simultaneously. However, Orlando would be a risky location for a mass production establishment.

Construction of the insectary in Florida would be especially expensive because of the elaborate precautions necessary to prevent escape of normal flies. The breeding stock constantly on hand would be one million normal adults. In addition, there would be 50,000,000 normal larvae produced each week. Grown screw-worm larvae are especially difficult to confine.

In addition to construction problems, a Florida location would require special personnel handling procedures. The employees would have to be issued special clothing for wear within the rearing establishment and much time of each 8-hour shift would be taken away from production for practice of safety precautions such as showering, changing clothes, and inspection.

If the sterilized fly plant should be built in the vicinity of San Antonio, Texas, these problems would be eliminated. Screw-worms occur in that area all the warm months of the year. Normal precautions would be taken to prevent releasing screw-worms to add to the native screw-worm population. However, the accidental escape of a few flies would not be serious. In Florida the accidental release of just one fertilized female near the end of the campaign could start a reinfestation all over again.

Shipping sterilized pupae by air from San Antonio to Orlando would be partly paid for by rail freight savings on meat and blood. If rearing was done at Orlando, ground hearts would need to be shipped in from locations such as Ft. Worth, Kansas City, or Chicago and blood from Ocala, Jacksonville, and Tampa. At San Antonio, local slaughters would provide all the blood required and more than enough cattle are slaughtered in Texas packing plants to provide all the ground hearts needed.

Fifty million pupae would weigh about 11,000 pounds. Packed in release containers the weight might be about doubled so it is estimated that 22,000 pounds would need to be shipped each week. The insects could be sent either in daily shipments of about 7,000 pounds.

The pupae would be loaded into the release tubes as they come out of the gamma sterilizing room. Daily scheduled shipments would reach Orlando about 24 hours before the first flies were due to emerge. The packages would be at a variable temperature ($80^{\circ} + - 10^{\circ}\text{F.}$) in transit but at Orlando they would be held for emergence in a room air conditioned to 80°F. and 90 percent relative humidity.

*Inform the man to prepare
release and answer questions*

-22-

The newly emerged flies would be sent each day by truck to the
on dispersal
15 air fields/which the/planes would be based.

Screw-worm survey during an eradication campaign.

In order to evaluate the progress of an eradication campaign
screw-worm activity will have to be constantly studied. This
would be done by 25 survey men (GS-5 Biological Aids) each assigned
a 2,000 square mile area.

Within his assigned area the survey man would work with county
agents, veterinarians, farmers, and ranchers to perform a dual job.
He would encourage animal management which would avoid unnecessary
wounds, and assure insecticide treatment of any abrasions to
prevent infestation. He would constantly solicit farmer and rancher
cooperation to prevent screw-worm breeding in neglected domestic
animals.

In the course of his work the survey man would make regular
notes on screw-worm case incidence in livestock and pets. He
would take samples of larvae for identification by specialists and
he would collect egg masses whenever he had opportunity

Each survey man would also operate 4 liver baited status traps,
which he would service weekly. He would save trapped flies, larvae
and egg masses for identification by the technical records team.

As instructed by his superior the survey man would park at
designated times at check points to note the course and timing of
the contract airplane scheduled to be flying over on a fly
dispersal course.

There would be two (GS-7 Entomologists)/^{assistant} survey supervisors.

One assistant supervisor would be responsible for surveys south of Orlando and the other would watch over surveys in the northern half of the area. The assistant survey supervisors would travel constantly over their assigned areas, checking with the work of the local survey men. There would be one survey supervisor (GS-9 Entomologist) in general charge of surveys. He would designate locations for all status traps. He would travel frequently over the entire state checking survey operations. He would also travel north of Florida determining area of summer migration and would establish whether there was any winter fly activity north of the treated area.

Technical field records during an eradication program

Most of the adult screw-worm flies in nature are a bright blue and are somewhat more robust than screw-worms reared in the laboratory. An experienced entomologist can usually distinguish between wild and released flies. Thus status trap catches determined by an experienced worker will give an index of the native population. The traps will also give an idea of migration and survival of the released sterilized flies.

The larvae of several species of common blowflies are often found in wounds and mistaken for screw-worms. An entomologist who knows larvae characters can quickly distinguish screw-worms from other species of maggots.

Therefore it is vital to have an identification team that can determine screw-worms with certainty to keep accurate records based on correct determinations. It is proposed that 10 GS-7

entomologist working under a GS-9 assistant supervisor and a GS-11 supervisor have the responsibility of insect identification.

The 10 GS-7 entomologist would have the additional responsibility of collecting screw-worm egg masses and determining which are fertile and which are not (hence laid by flies mated to sterile males.)

The egg mass collectors would travel over Florida in pick up trucks each equipped with a portable 6' x 8' aluminum panel goat pen. A goat pen would be set up in a tent location for a day and stocked with 3 wounded goats (which had been previously infested with screw-worms for 3 days in a locked security pen). The pens would be set up early in the morning and before dark the entomologist would visit the pens, collect any egg masses and hold them on moist filter paper in petri dishes for hatching records to be taken the following day.

The field entomologist would work out of the county agent's office in each county he visited. The survey men would leave their status trap catches and larval and egg collections for his determination at the county agent's office.

The 10 entomologist would be sent to whatever counties the supervisor considered most important for egg mass data. The supervisor and his assistant would remain at the central headquarters maintaining records, determining flies sent in from the field, and checking on the determinations of the field crews.

Two biological aids (GS-3) would be at the headquarters, infesting and caring for goats to provide the field entomologists with healthy attractive replacement animals which would be changed twice each week. The aids would also assist the supervisors by unpacking and sorting status trap collections sent in from the field.

Procedures to start an eradication program.

Assuming that an eradication program is approved and Congress votes that it become available July 1, 1957 the following timetable is proposed:

A. Further research:

July 1, 1957, with the existing screw-worm staff at the Orlando, Florida, laboratory, cooperating with engineers and pilots of the Aircraft Equipment Center, start expanded field tests in Florida. The greatest need is for information on swath width between flight lanes, time lapse between releases and development and testing of automatic release equipment.

These points must be established in large scale field trials in order to contract most economically for fly dispersal services.

Insects could be reared and sterilized with facilities now available at Orlando.

With two months' notice and adequate funds for expansion it will be possible to rear and sterilize from 2 to 10 million flies weekly at Orlando for use in large scale field trials. This means that Orlando can furnish test insects and field entomologist observers whenever the engineers have equipment ready for testing.

The distribution studies and dispersal equipment development should continue right to the start of the large scale releases of the eradication campaign. In addition to gathering information on most economical methods of distributing flies, the large scale testing with sterilized flies should depress native screw-worm population in the test areas. That should be of immediate benefit to livestock growers and would help lower the population prior to the full scale eradication effort.

B. Construction:

1. Orlando, Florida

July 1, 1957 - make detailed plans for sterilized pupa receiving and fly emergence room. If suitable space cannot be located for lease, a building should be constructed to be ready by July 1, 1958.

2. At 15 Florida airports

July 1, 1957 - two 10 X 14 fly holding rooms air conditioned to 80°F. and 80% relative humidity need to be located. If facilities/^{are}not available for rent they will have to be built at each airport. At 3 South Florida airports facilities need to be ready by Sept. 1, 1957 for holding flies for field trials. At remaining airports in the southern half of Florida facilities ~~and~~ should be ready by July 1, 1958. In northern Florida, facilities should be ready by January 1, 1959.

3. At San Antonio or Kerrville, Texas.

July 1, 1957.- Select plant location and begin preparation of detailed plans for big production plant.

September 1, 1957 - Advertise for bids due Oct. 15 for construction to start Dec. 1, and be completed in 4 months.

April 1, 1958 - Rearing plant building should be completed and installation of equipment begin.

July 1, 1958 - All equipment should be installed and tested during month.

July 1, 1958 - Plant should be in condition for a trial run.

August 1, 1958 - Plant should be ready to start production of sterilized flies.

C. Bids for major supplies and services.

July 1, 1957 - Advertise for bids for delivery of 75,000 lbs. ground heart each week through June 30, 1958 to Orlando, laboratory.

November 1, 1957 - Advertise for bids on fly dispersal contracts estimated for January 1 to June 30, 1958.

January 1, 1958 - Advertise for bids on air delivery of flies from Texas plant to Orlando, Florida beginning August 1, 1958 with shipment of 10,000,000 pupae and

increasing by 10,000,000 each month until full schedule is reached December 1, 1958.

January 1, 1958 - Advertise for bids on delivery of meat and blood to Texas plant in weekly shipments beginning July 1, 1958. Delivery to be at 15,000 lbs per week in July, 30,000 in August, 45,000 September, 60,000 in October and 75,000 in November and each succeeding month. (Advertise for delivery of blood in proportional amounts.)

January 1, 1958 - Establish specifications and advertise for bids on full scale fly services to start August to November 1958.

Quarantine Procedures.

No quarantine measures would be necessary at the start of the campaign but possibly by November 1, 1958 that part of Florida south of Lake Okechobee might be free of flies. As soon as field data indicate that native fly breeding is under control in an area there should be some restriction on shipping in cattle which are likely to be infested.

The problem will first arise as a local one within the State of Florida and could be handled by appropriate state legislation. There should be legislation prohibiting the shipping of livestock into or through a screw-worm free area if the shipment originates in or passes through infested territory.

An exception could be made for livestock properly sprayed at the start of a trip with a designated screw-worm preventive. For example, the past years work at the Kerrville laboratory suggests that cattle and sheep can be protected from infestation for at least 10 days by spraying them with a new phosphorus insecticide, Bayer 21/199.

If it were required that cattle shipped into a quarantine zone had to have a veterinarians certificate that they were not infested and had been sprayed with a preventive treatment, this should greatly cut down the risk of reintroduction of screw-worms.

It is possible that during an unusually warm winter, eradication in Florida could be successful but flies might survive in Georgia and Alabama. In such a case Florida would have to be protected against screw-worm importations from those states.

Once the southeast is freed of screw-worms the permanently protected area would comprise the states of Alabama, Florida, and Georgia. It would seem simplest for those three states to assume most of the responsibility for protecting themselves. This could be done by requiring a veterinarians certificate of inspection and spraying for all cattle imported from screw-worm areas.

The USDA could help by providing a relatively inexpensive screw-worm survey service. A permanent team of 4 survey men would be needed. Two men could be assigned to the Southwest. They would map the overwintering area in Texas and follow the annual summer migration of the flies furnishing weekly maps of the spread of the infestation. In the fall and winter they would similarly trace the

reduction of screw-worms as cold limited the infestation southward.

Since screw-worm flies look much like ordinary C. moulloria , blowflies, and as several species of carcass-breeding maggots occasionally grow in wounds; there will always be alarms that screw-worms have returned to the Southeast. These reports should not be discounted but they should be investigated immediately. The Southeastern work will require the services of two men, systematically checking status traps, conferring with county agents, livestock growers, and agricultural officials, and when rumors of screw-worms are heard--checking to see whether the infestation is genuine.

Prompt detection of a new screw-worm infestation might enable its eradication, quickly and economically.

The above program will not positively protect against reintroduction of screw-worms but it should greatly reduce the chances of accidental importation.

It may be pertinent to mention that there are large annual ^{infested} shipments of cattle from screw-worm/areas of Texas to summer pastures and feed lots in South Dakota, Iowa, and Illinois. At present there are no special anti-screw-worm precautions yet outbreaks in those states are rare. Those three states are mentioned because they have had outbreaks once or twice in history. Most of the northern states have never had screw-worm outbreaks.

Therefore, with proper cooperation between state and federal regulatory services the Southeast should have an excellent chance of remaining free of screw-worms at a very modest preventive cost.

